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Action Concepts in the Brain: An Activation Likelihood Estimation Meta-Analysis

Abstract

Many recent neuroimaging studies have investigated the representation of semantic memory for actions in the brain. We used activation likelihood estimation (ALE) meta-analyses to answer two outstanding questions about the neural basis of action concepts. First, on an “embodied” view of semantic memory, evidence to date is unclear regarding whether visual motion or motor systems are more consistently engaged by action concepts. Second, few studies have directly investigated the possibility that action concepts accessed verbally or nonverbally recruit different areas of the brain. Because our meta-analyses did not include studies requiring the perception of dynamic depictions of actions or action execution, we were able to determine whether conceptual processing alone recruits visual motion and motor systems. Significant concordance in brain regions within or adjacent to visual motion areas emerged in all meta-analyses. By contrast, we did not observe significant concordance in motor or premotor cortices in any analysis. Neural differences between action images and action verbs followed a gradient of abstraction among representations derived from visual motion information in the left lateral temporal and occipital cortex. The consistent involvement of visual motion but not motor brain regions in representing action concepts may reflect differences in the variability of experience across individuals with perceiving versus performing actions.

Disciplines

Nervous System | Neurology | Neuroscience and Neurobiology | Neurosciences

Remyelination reporter reveals prolonged refinement of spontaneously regenerated myelin

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Neurological diseases and trauma often cause demyelination, resulting in the disruption of axonal function and integrity. Endogenous remyelination promotes recovery, but the process is not well understood because no method exists to definitively distinguish regenerated from preexisting myelin. To date, remyelinated segments have been defined as anything abnormally short and thin, without empirical data to corroborate these morphological assumptions. To definitively identify regenerated myelin, we used a transgenic mouse with an inducible membrane-bound reporter and targeted Cre recombinase expression to a subset of glial progenitor cells after spinal cord injury, yielding remarkably clear visualization of spontaneously regenerated myelin *in vivo*. Early after injury, the mean length of sheaths regenerated by Schwann cells and oligodendrocytes (OLs) was significantly shorter than control, uninjured myelin, confirming past assumptions. However, OL-regenerated sheaths elongated progressively over 6 mo to approach control values. Moreover, OL-regenerated myelin thickness was not significantly different from control myelin at most time points after injury. Thus, many newly formed OL sheaths were neither thinner nor shorter than control myelin, vitiating accepted dogmas of what constitutes regenerated myelin. We conclude that remyelination, once thought to be static, is dynamic and elongates independently of axonal growth, in contrast to stretch-based mechanisms proposed in development. Further, without clear identification, past assessments have underestimated the extent and quality of regenerated myelin.

regeneration | plasticity | internode

Nervous system disorders including traumatic injury, stroke, and neurodegenerative diseases such as multiple sclerosis induce loss of myelin and myelinating cells, interrupting signal conduction and depriving axons of trophic support essential for survival (1–4). Postmitotic oligodendrocytes (OLs) do not readily participate in remyelination (5, 6). Instead, glial progenitors, distinguished by expression of the α -receptor for PDGF and the chondroitin sulfate proteoglycan neural/glia antigen 2 (NG2) proliferate following demyelination and differentiate into remyelinating cells within a few weeks (7–10). Regeneration of myelin membranes restores saltatory conduction and supports axonal integrity, leading to partial recovery of function (3, 4, 11, 12). However, remyelination can fail during disease progression, and limited or abnormal myelin regeneration is thought to underlie chronic conduction deficits following trauma (11, 13, 14). Enhancing or substituting endogenous remyelination via pharmacological intervention or stem/progenitor cell transplantation has been a major, but unrealized, focus of clinical therapy development for decades (15–17).

There is much we do not understand about spontaneous remyelination, including the rate of OL regeneration, whether remyelinating cells select specific phenotypes or morphotypes of axons to ensheath, and whether the initial number, thickness, or length of myelin internodes are dynamic. This is partly because no methods exist to definitively discriminate spontaneously regenerating myelin membranes from spared or degenerating sheaths. There are also no known molecular markers for regenerating myelin. Much of our understanding of myelin replacement is instead derived from interpretation of small-field EM or quantification of indirect histo-

logical markers (14, 18–21). Groundbreaking EM studies in the 1960s and 1970s confirmed the phenomenon of central remyelination, revealing thin, loosely wrapped sheaths that resembled immature myelin (18) as well as abnormally short myelin internodes (20) a few weeks after demyelination. Since that time, abnormally thin or short sheaths have been presumed to represent regenerated myelin. Investigators still commonly use these morphological characteristics alone to identify and study remyelination (11, 19, 20, 22).

This approach has likely limited understanding of the endogenous regeneration process. Measuring sheath length or thickness does not empirically determine whether a myelin sheath is new, degenerating, or preserved. Inappropriately thin and shortened myelin segments also result from partial demyelination of developmentally generated sheaths (23–25). Furthermore, it has long been suspected that internodes with normal proportions could be formed by remyelinating cells (19). If regenerated sheaths were able to thicken or elongate over time, for example, they would be morphologically indistinguishable from normal myelin. In short, we cannot be sure that all abnormally thin or short myelin is regenerated, nor that all sheaths with normal dimensions are a product of development rather than regeneration. Myelin sheath dimensions affect signal conduction speed considerably, and sluggish conduction following insult has been attributed in part to irregular remyelination (11, 12, 26–29). A better comprehension of these fundamentals is essential in guiding a new understanding of spontaneous myelin regeneration and therapeutic approaches to demyelinating disorders.

To follow the evolution and maturation of regenerating myelin membranes, we used a model of spinal cord contusion injury known to cause significant partial demyelination of spared axons followed by spontaneous remyelination (28). We targeted reporter expression to the membranes of proliferating NG2⁺ glial progenitors via postinjury retrovirus injections in a mouse strain that ubiquitously expresses membrane-bound GFP following Cre recombination (30). Our findings revealed dynamic remodeling of myelin internodes over time and challenge several morphological dogmas in regard to how regenerated myelin forms.

Results

Cre-Mediated Recombination Is Evident in Premyelinating Glial Progenitors Acutely After Injury. To generate a reporter system for remyelination, three essential elements were combined (Fig. S1). (i) Remyelinating cells were specifically targeted by a combination of the NG2 promoter (31) and a retrovirus that integrates DNA into the host genome only during mitosis (32). We chose the NG2 promoter because it is expressed by resident glial progenitors that can produce OLs and Schwann cells (SCs) after injury as well as by infiltrating peripheral SCs that participate in central remyelination

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(10, 33–35). The promoter and its efficient targeting of NG2⁺ glial progenitors has been fully characterized previously (31). (ii) We tracked the remyelination process over a long postinjury period by using the Cre-loxp system to induce stable reporter gene expression. (iii) Clear visualization of myelin membranes was accomplished by using a membrane-targeted reporter, as most cytoplasm is extruded from compact myelin (36). We used a mouse strain that ubiquitously expresses membrane-targeted tandem dimer Tomato (mT) but switches to membrane-targeted GFP (mG) following Cre-mediated recombination (30). We injected virus into spinal cords of reporter mice 6 d after moderate thoracic contusion injury, when many NG2⁺ progenitors that go on to remyelinate are thought to proliferate (15, 34).

To ensure that virus targeted proliferating glial progenitor cells rather than spared, postmitotic OLs, we examined spinal cord sections of contused mice for mG expression 5 d post virus (DPV) injection. At this time point, recombination in infected cells should have induced a switch from mT to mG expression (30), but myelin regeneration was unlikely to have begun (18). We therefore expected to find mG⁺ cells, but not mG⁺ myelin sheaths. Numerous perilesional mG⁺/mT⁺ cells were observed at 5 DPV (Fig. 1A). Some mG⁺ cells were highly NG2-immunopositive and exhibited characteristic OL progenitor cell morphology (Fig. 1B). As expected, no mG⁺ myelin was observed at 5 DPV. To further establish that mG⁺ populations were not composed of postmitotic cells and eliminate the possibility that preexisting myelin was labeled, animals received a single i.p. injection of the mitotic marker BrdU concurrent with virus injection. We found at 5 DPV that mG⁺ cells were BrdU⁺, indicating that they had undergone division at the time of virus injections (Fig. 1C).

We examined tissue sections at 14 DPV to determine whether cells labeled 6 d postinjury had commenced regeneration of myelin. mG⁺ cells showed evidence of differentiation: they exhibited complex multiprocess morphologies and APC⁺ cell bodies characteristic of premyelinating OLs (Fig. 1D). However, no mG⁺ myelin was visible at 14 DPV. Taken together, these results confirm that the virus infected dividing NG2-expressing glial progenitors, and not postmitotic, myelinating OLs. Furthermore, we confirm that regenerated myelin membranes take at least 2 wk to initially form (18).

Regenerating Myelin Sheaths Are Evident 1 mo After Virus Injection.

To determine whether the reporter system targeted regenerating myelin membranes, we examined longitudinally sectioned spinal cords of injured mice killed at 1 mo post virus (MPV) injection,

when remyelination was predicted to be well under way (18). Sections contained OL-associated mG⁺ sheaths (Fig. 2), which were hollow as expected (Fig. 2D) and filled with neurofilament (NF)⁺ axons (Fig. 2F). Sheaths exhibited contactin-associated protein 1 (CASPR1)⁺ paranodes at each end, indicating a reconstituted nodal structure (Fig. 2F). GFP signal within OL-regenerated sheaths colocalized with the marker of compact myelin, myelin basic protein (MBP; Fig. 2E). OL somata were remotely connected to multiple myelin sheaths by fine processes. The myelin reporter allowed us to map many of the mG⁺ myelin sheaths to mG⁺ cell bodies immunopositive for the OL marker APC (Fig. 2C). Physical parameters (length and thickness) of mG⁺ regenerated myelin were quantified by confocal microscopy (Figs. 3 and 4). Other mG⁺ internodes exhibited an mG⁺ soma with DAPI⁺ nucleus juxtaposed to the exterior of each sheath (Fig. 5A and B) and immunopositivity for P0 and periaxin (Fig. 5D and E), characteristic of peripheral myelin produced by SCs.

By using confocal microscopy, we observed coincident but also nonoverlapping regions of mG and MBP indicating reporter labeling of noncompact and compact myelin. To determine whether reporter-labeled regenerated myelin was multilamellar and compact, we performed immuno-EM on tissue sections stained with an anti-GFP primary, followed by a secondary antibody conjugate made with quantum dot nanocrystals (Qdots; ~4 × 10 nm in length) that fluoresce under UV light and are also visible by EM. We confirmed GFP/Qdot colocalization by confocal microscopy (Fig. 2G) and visualized Qdots of the appropriate size (4 × 10 nm) labeling compact, multilamellar myelin in injured mice at 3 MPV (Fig. 2H–L).

Quantitative Characterization of Myelin Sheaths in Uninjured Controls.

To establish a baseline for our experiments in the injury model, we quantified the structural characteristics of normal myelin in uninjured reporter mice. Animals received a single thoracic microinjection of virus at postnatal day (P) 4 or P5, when many NG2⁺ progenitor cells undergo proliferation before differentiating into myelinating cells. Animals were killed as age-matched, uninjured adult controls. They exhibited mG⁺ myelin sheaths bounded by CASPR1⁺ paranodes throughout the thoracic white matter (Fig. 3A). We quantified myelin dimensions by using confocal microscopy. The mean internode length of developmentally generated myelin in thoracic segments 8 to 11 was 214 ± 97 μm, with a range of 28 to 495 μm (Fig. 3B). Consistent with previous EM studies (20, 37), sheath length and width were positively correlated (Fig. 3E). We also determined g-ratio, the relationship of axonal diameter to

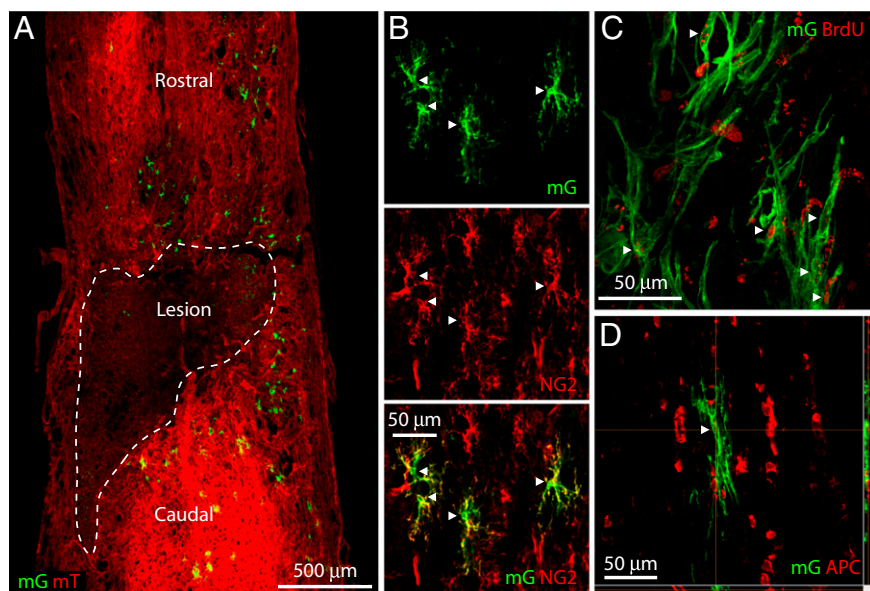
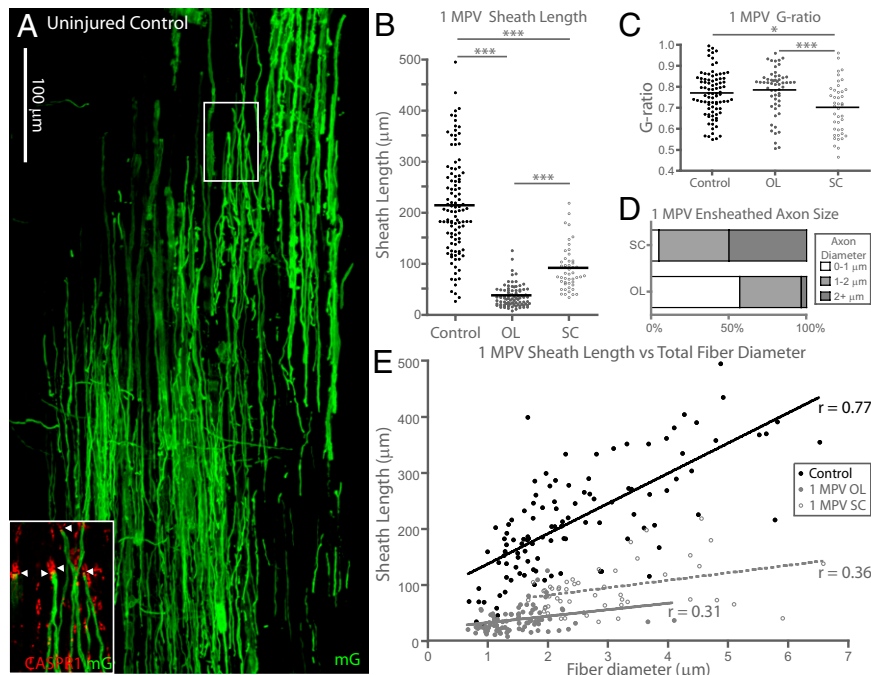


Fig. 1. Cre-mediated recombination is evident in glial progenitors, but not in myelin early after injury, demonstrating targeted labeling of remyelinating cells, and not preexisting myelin. (A) A low-power, merged confocal z-series depicts perilesional mG⁺ cells 5 DPV, but, as expected, no mG⁺ myelin sheaths are yet evident. (B) Some mG⁺ cells are highly NG2⁺ with classic glial progenitor morphology (arrowheads). (C) mG⁺ cells exhibited BrdU⁺ nuclei 5 DPV (arrowheads). More than 80% were BrdU⁺, although some stained weakly, indicating likely BrdU dilution with multiple cell divisions. Cells in C resemble immature SCs. (D) At 14 DPV, still no mG⁺ sheaths were evident, but many cells exhibited complex elongated morphology and APC⁺ somata characteristic of premyelinating OLs.



packaging cells (American Type Culture Collection). Infectious virus particles were harvested 48, 72, and 96 h posttransfection and concentrated via ultracentrifugation to 1×10^{10} infectious particles per microliter.

Confocal Microscopy and Quantitative Analysis. Multichannel confocal z-series images were generated using a Nikon A1 Confocal System attached to a Ti-E inverted microscope platform. To quantify myelin and axonal dimensions, all mG^+ sheaths within six longitudinal sections containing the lesion from each animal were imaged ($n = 2$ each from ventral, central, and dorsal spinal cord). Quantification was performed by using NIS Elements software (Nikon). Sheath length was measured along NF^+ axons from one CASPR^+ paranode to the other. Sheath widths were measured in at least four locations in flattened z-stacks and averaged. Basal lamina, but not cell body, was included in SC sheath width measurements. SC sheaths were identified by the presence of an mG^+ soma with DAPI^+ nucleus hugging the exterior of the sheath. OL sheaths were identified by the presence of attached thin projections from a satellite $\text{mG}^+/\text{DAPI}^+$ cell body. NF^+ axonal diameter was measured in at least two locations and averaged. Where NF antibody penetration was weak (typical within the internode in heavily myelinated regions), average width of both CASPR -labeled paranodes was taken as a measure of axonal diameter.

Immunoelectron Microscopy. Tissues were prepared as described previously (28). Briefly, three injured mice were killed and perfused at 3 MPV with

ice-cold 3% (wt/vol) paraformaldehyde/0.5% glutaraldehyde. All steps were performed on ice. Coronal sections of spinal cord 80 μm thick were made on a Vibratome. Sections were rinsed in PBS solution, blocked in 2% BSA in PBS solution, and incubated with rabbit anti-GFP (1:500; Millipore) in block for 2 h followed by goat anti-rabbit Qdot 605 (1:100; Invitrogen) for 1 h in block, and rinsed in PBS solution. Some sections were imaged immediately on a confocal microscope to confirm colocalization of GFP with Qdots. Sections were then dehydrated and processed for EM as previously. Ultrathin sections were imaged by using a JEM-1400 transmission electron microscope (JEOL) with a Gatan Ultrascan 1000XP camera.

Statistical Analysis. Statistical significance among groups was determined by one-way ANOVA with Bonferroni multiple comparison posttest for three or more groups or two-tailed, unpaired t test for two groups. Pearson correlations were used to determine the relationship between sheath length and axon or total fiber diameter. A CI of 95% and Prism software was used for all comparisons (GraphPad). Results in the text are reported \pm SD.

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Supporting Information

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SI Methods

Contusion Spinal Cord Injury. Female mice aged 3 mo were anesthetized via i.p. injection of ketamine (100 mg/kg; Fort Dodge Animal Health) and xylazine (20 mg/kg; Lloyd Laboratories). The skin of the back was shaved and scrubbed with Betadine and ethanol. s.c. local anesthetic agent (lidocaine and bupivacaine 1 mg/kg each; Hospira) was injected, the skin was opened, and a dorsal laminectomy was performed at thoracic segment (T) 9 or T10. An impact probe was lowered onto the dural surface and displaced by 0.43 mm (i.e., moderate injury) by using The Ohio State University contusion device. Gelfoam was placed on the dural surface, and muscle and skin were closed in layers. Animals were kept on a heating pad (38 °C) until fully awake. s.c. warm Ringer solution was given for hydration, and gentamicin (4.8 mg/kg; APP) and twice-daily buprenorphine (0.05 mg/kg; Reckitt Benckiser) were administered for 7 and 2 d, respectively. Manual bladder expression was performed twice daily until voluntary control returned.

Virus Microinjection. Six days postcontusion, animals were anesthetized as described earlier, and the laminectomy site was reexposed. Two 2- μ L injections of virus were made into the dorsal columns 0.5 mm rostral and 0.5 mm caudal to the lesion epicenter at a rate of 0.5 μ L/min. The micropipette was slowly extracted after 2 min. Wound closure and postoperative care were as described earlier. Animals received s.c. warm Ringer solution and buprenorphine and i.p. BrdU (10 mg/mL).

Uninjured control animals ($n = 3$) received a 1- μ L spinal microinjection of virus to label developmentally generated myelin. Postnatal day 4 to 5 pups were anesthetized on ice for 5 min and maintained on an ice pack during injection. Skin of the back was sterilized as described earlier, and a hemilaminectomy was performed at T9 or T10. Virus was injected just lateral of the dorsal spinal artery at a rate of 0.5 μ L/min at 0.1 to 0.2 mm below the dural surface, and the micropipette was extracted after a 2-min wait. Gelfoam was placed on the dural surface, and the

skin was closed with Vetbond. Pups were kept on a heating pad (38 °C) until fully awake. Pups received s.c. buprenorphine (0.03 mg/kg) and were returned to the nest. They were allowed to mature until euthanasia at age 3 to 4 mo.

Tissue Preparation. Mice were given an overdose of Beuthanasia-D (Schering-Plough) and intracardiac perfusion with Ringer solution to exsanguinate, then with 4% paraformaldehyde. Injured mice were killed at 5 d post virus injection (DPV; $n = 4$), 14 DPV ($n = 4$), 1 MPV ($n = 5$), 3 MPV ($n = 5$), or 6 MPV ($n = 5$). Spinal cords were postfixed in 4% paraformaldehyde overnight, equilibrated in 30% sucrose for cryoprotection, frozen in Optimum Cutting Temperature medium (Ted Pella), and sectioned longitudinally onto slides at 20 to 40 μ m.

Immunohistochemistry. Slides were rinsed with PBS solution for 10 min and blocked for 60 min in PBS solution with 5% donkey serum (Jackson ImmunoResearch) and 0.5% Triton X-100 (Amresco). Sections were incubated for 24 to 48 h at 4 °C in block with primary antibodies (as detailed later), then at 4 °C overnight in block with appropriate secondary antibodies: anti-mouse, anti-chicken, anti-rat, or anti-rabbit 647 or 594 (1:300; Invitrogen). Tissue was rinsed five times for 10 min each in PBS solution. The last rinse included the nuclear marker DAPI (1:1,000). Slides were coverslipped with Gelvatol. Primary antibodies included rabbit anti-neural/glial antigen 2 (1:500; gift of W. Stallcup, Sanford-Burnham Medical Research Institute, La Jolla, CA), rat anti-BrdU (1:500; Novus Biologicals), pan-axonal neurofilament mouse anti-SMI-312R and -311R (1:500; Covance), rabbit anti-contactin-associated protein (1:100; gift of S. Trimmer, University of California, Davis, CA), mouse anti-APC-CC1 (1:200; Calbiochem/EMD Biosciences), rabbit anti-myelin basic protein (Millipore), and chicken anti-P0 (1:100; AvesLabs) or rabbit anti-periaxin (1:2,000; gift of P. Brophy, University of Edinburgh, Edinburgh, United Kingdom).

